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Effects of Valine, Leucine, Isoleucine, and a Balanced Amino Acid Solution on the Seizure Threshold to Picrotoxin in Rats

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SKEIE, B., A. J. PETERSEN, T. MANNER, J. ASKANAZI AND P. A. STEEN. *Effects of valine, leucine, isoleucine, and a balanced amino acid solution on the seizure threshold to picrotoxin in rats.* PHARMACOL BIOCHEM BEHAV **48**(1) 101–103, 1994.—During infusion of branched-chain amino acids (BCAAs) in humans, changes in ventilatory drive, sleeping pattern, and appetite have been reported. The mechanism by which BCAA exerts their effects on CNS remains unclear. An infusion of a BCAA solution (300 mg/kg) has previously been found to increase the seizure threshold in rats to the proconvulsant drug picrotoxin, an antagonist on the GABA-benzodiazepine receptor complex. In this study, each of the BCAAs given separately (valine, leucine, isoleucine; 300 mg/kg) (n = 10) increased the mean latency time to onset of seizures vs. placebo as an indication of an increased seizure threshold. A balanced amino acid solution (Vamin-Glucose®) had no effect on the seizure threshold. Thus, these CNS effects are specific for BCAAs and occur with all three.

Branched-chain amino acids GABA-receptor complex Proconvulsant drugs Nutritional pharmacology

THE branched-chain amino acids (BCAAs), valine, leucine, and isoleucine, appear to have effects on the CNS. In humans we observed changes in the ventilatory drive, sleep pattern, and appetite/food intake during infusion with BCAA (5,7, 15,16). Intake of BCAAs during prolonged exercise has been found to improve mental and physical performance (2). A reduction in plasma levels of BCAAs is observed in the encephalopathy seen in chronic liver disease as well as a result of critical illness (17). Patients in hepatic coma awake at least as quickly in response to intravenous administration of BCAA and hypertonic dextrose solution as they do in response to conventional treatment, and both regimens work quicker than placebo (9).

The mechanisms responsible for these CNS effects remain unknown. A change in the balance of plasma amino acids may lead to alterations in the concentration of neurotransmitters in the brain. Thus, it has been suggested that an increased concentration of the BCAA in plasma will decrease the rate and synthesis of 5-HT (from tryptophan) into the synapse, so that a change in brain functions results (3).

GABA is the major inhibitory neurotransmitter in the brain. The heterogenous GABA-receptor exists in most inhibitory synapses in the CNS and can be regulated by both benzodiazepines (BDZs) and barbiturates. High-affinity recognition sites for BZDs are part of the GABA receptor complex on the plasma membrane of neurons in the brain (8,11). BDZs agonists promote GABAergic neurotransmission, and, hence, the hypnotic and anxiolytic effects of this class of drugs, by binding to these sites (10).

The proconvulsant drug picrotoxin acts as an antagonist on the GABA-BDZ receptor complex (12). We have observed an increase in the seizure threshold to picrotoxin in rats given a mixture of the three BCAAs (valine, isoleucine, leucine) when compared to placebo (14). We wondered if this was a general effect of BCAAs or specific for one of these three amino acids. Thus, in the present study we have evaluated the

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TABLE 1

MEAN LATENCY TIME ± SD FROM INJECTION OF PICROTOXIN TO ONSET OF SEIZURES

	Latency Time in Min
Leucine	11.5 ± 1.9*
Valine	11.5 ± 1.4*
Isoleucine	11.6 ± 1.8*
Amino acid solution	8.8 ± 1.5
Placebo	9.5 ± 1.3

$n = 10$.
*$p < 0.05$ compared to placebo.

effect on the seizure threshold of each of the BCAAs given separately compared to a balanced amino acid solution and placebo.

METHOD

Fifty male Wistar rats were weighed and randomized to receive either an IP injection with valine (Sigma Chemical Co., St. Louis, MO) (2.5%, 300 mg/kg) ($n = 10$), leucine (Sigma) (2.5%, 300 mg/kg) ($n = 10$), isoleucine (Sigma) (2.5%, 300 mg/kg) ($n = 10$), a balanced amino acid solution (Vamin-Glucose®, Kabi Pharmacia, Sweden) (7%, 300 mg/kg) or placebo (saline, 3 ml) ($n = 10$). After 120 min, rats received an IP injection with picrotoxin (10 mg/kg) (Sigma). The latency time from injection of picrotoxin to onset of tonic seizures was recorded. The observer did not know what pretreatment the rat had received. After onset of seizures the rats were given a lethal injection of thiopentone IP. Statistical analysis was made using ANOVA and Student's t-test. $p < 0.05$ was considered statistically significant.

RESULTS

The results are listed in Table 1. There was a significant increase in the latency time to onset of seizures in the three groups pretreated with the BCAAs leucine, valine, and isoleucine when compared to the balanced amino acid solution and placebo. There was no difference between the results with the BCAAs, nor between the balanced amino acid solution and placebo.

DISCUSSION

The use of the physiological effects of certain nutritional components as therapy has attracted growing attention and the field of nutritional pharmacology is evolving (4). The BCAA have interesting physiological properties with stimulatory effects that may be pharmacologically valuable. The effects of BCAAs in hepatic encephalopathy (17), their stimulatory effects on ventilation and appetite/food intake (5,6), as well as the improvement in sleep pattern (15,16) and mental and physical fatigue (2) observed during BCAA administration, suggest that a stimulation of the CNS occurs.

BCAA may influence serotoninergic neurotransmission. At the blood-brain barrier, the BCAAs compete with the large neutral amino acids, of which the aromatic amino acids are a subgroup, for transport into the brain, thereby limiting the entry of amino acids such as tryptophan (3). Amino acids, as well as being neurotransmitters in their own right, are also precursors for many of the neurotransmitters (e.g., the catecholamines, 5-HT, histamine, and the peptide neurotransmitters). The entry of the aromatic amino acids, including tryptophan, into the brain will be affected by the relative plasma concentrations of other large neutral amino acids, particularly the BCAAs.

Picrotoxin is a much used experimental proconvulsive agent that binds to the GABA-BZD receptor complex (12). In a previous study, we observed that BCAAs demonstrated an anticonvulsant effect when given to rats pretreated with picrotoxin (14). The present study shows that the effect on seizure threshold is observed after pretreatment with each of the BCAAs given separately. The latency time to onset of seizures was not longer for rats pretreated with a standard amino acid solution, indicating that the effect on seizure threshold is a specific property of the BCAAs.

An interaction between the BCAAs and the GABA-receptor complex could explain neuropharmacological effects observed during administration of BCAAs. We speculate if the increased seizure threshold to picrotoxin, a GABA-BZD receptor antagonist, indicates that the CNS effects of the BCAAs are mediated via the GABA-BZD receptor complex. This could be an indirect effect of the BCAAS due to a competition with other amino acids on the blood-brain barrier with alterations in the brain concentration of neurotransmitters or a direct action of BCAAs or their metabolites on the GABA-BZD receptor complex. However, this is still speculative. Studies are needed to test BCAAs against other proconvulsant agents acting at the GABA-receptor complex as well as against GABA-receptor independent convulsants to improve our understanding about the mechanisms for the BCAAs effects.

The increase in seizure threshold observed after administration of BCAAs may have therapeutic aspects. Further studies should be undertaken to look at dose-response curves, time of peak effect, and if BCAAs given in combination with common anticonvulsants potentiate their effects.

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